Cys Gly Tyr Pro Gly Ile Ser Pro Glu Glu Cys Ala Ser Arg Lys Cys Cys Phe Ser Asn Phe Ile Phe Glu Val Pro Trp Cys Phe Phe Pro Asn Ser Val Glu Asp Cys His Tyr

or a homologue thereof that

A) has [at least] six disulphide bonds that form two trefoil domains, where the 12 cysteines that form the six disulphide bonds are in the configuration 1-5, 2-4, 3-6, 7-11, 8-10 and 9-12, and

B) is encoded by a nucleic acid sequence that hybridizes under high stringency conditions to a nucleic acid sequence that encodes SEQ ID NO:1,

D3 Cont

wherein said polypeptide is characterized by being in N-glycosylated form and having spasmolytic activity.

Please add the following claims:

40. (New) The polypeptide of claim 27, wherein said polypeptide has an amino acid sequence according to SEQ ID NO:1.

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41. (New) A pharmaceutical composition comprising the polypeptide of claim 40 together with a pharmaceutically acceptable carrier or excipient.

REMARKS

The amendments to claim 27 find support in Figure 2 and on page 3, lines 6-9 of the specification. Added claims 40 and 41 are directed to a polypeptide having an amino acid sequence according to SEQ ID NO:1.

Following entry of the above amendments to the claims, claims 27-33, 36 and 40-41 are pending.

The specification has been amended to insert a SEQ ID NO (SEQ ID NO:7) at page 13, line 28 as requested by the Examiner and to change SEQ ID NOs:7-13 at page 13, line 30 to page 14, line 24 to SEQ ID Nos:8-14. The amendments to the specification are fully supported by the Sequence Listing presented with the application as originally filed.

As required by 37 C.F.R. 1.121, "marked-up" copies of the amendments to the specification and claims are appended to this Amendment.

Rejection Under 35 USC §112, first paragraph

The Examiner maintained the rejection of claims 27-33 and 36 as containing "subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." (page 2 of Office Action). In addition, the Examiner also applied the rejection to added claim 39 because the limitation "high stringency conditions" does not add further, meaningful structural limitations to the claimed invention. Applicants respectfully traverse this rejection.

Applicants note that in claim 27 as amended herein, the homologs of SEQ ID NO 1 in addition to having spasmolytic activity and being N-glycosylated like SEQ ID NO 1, must 1) have six disulphide bonds that form two trefoil domains, where the 12 cysteines that form the six disulphide bonds are in the configuration 1-5, 2-4, 3-6, 7-11, 8-10 and 9-12, and 2) be encoded by a nucleic acid sequence that hybridizes under high stringency conditions to a nucleic acid sequence that encodes SEQ ID NO:1. For the reasons set forth below, it is Applicants' position that the above functional and structural requirements place the claims in compliance with the written description requirement of §112, first paragraph.

First, in response to the Examiner's assertion that the limitation "comprising at least six disulfide bonds that form two trefoil domains " sets forth only minimal structural limitations and does not set forth the required configuration of the six disulfide bonds, Applicants note that claim 27 as amended sets forth the required configuration of the six disulfide bonds. Further, as noted in the previous Amendment, the presence of two trefoil domains in the claimed polypeptides provides a common structural attribute that serves to distinguished the claimed polypeptides from other polypeptides and as indicated on page 5, line 24-26, it is the trefoil domain structure encompassed by the term "homologue" in amended claim 27 that gives the claimed human spasmolytic polypeptide its biological activity (ie spasmolytic effect).

Second, in response to the Examiner's assertion that the limitation "high stringency conditions" does not add further, meaningful structural limitations to the claimed invention, Applicants submit that the Patent Office's guidelines for examination of patent applications under 112, first paragraph written description requirement expressly state that the written

description requirement can be met by "showing that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics ....ie complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics" 66 Fed. Reg. at 1106, emphasis added (copy attached). For example, the Patent Office would find compliance with the written description requirement for a claim[1] to a genus of nucleotides that hybridize with a specific sequence (ie SEQ ID NO 1) "since highly stringent hybridization conditions in combination with the coding function of DNA and the level of skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention" see Example 9 of the Written Description Guidelines at pages 36-37, available at http://www.uspto.gov/web/patents/guides.htm. In particular, the Guidelines state that "a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because the highly stringent hybridization conditions set forth in the claim yield structurally similar DNAs" Guidelines at page 36.

Here, the specification discloses that the term "homologue" indicates "a polypeptide encoded by DNA which hybridizes to the same probe as the DNA coding for HSP under conditions of high or low stringency (eg as described in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989)" (page 3, lines 5-11 of application) and the disclosure of hybridization and wash conditions based on the T_m of a hybrid formed between a probe and its target as set forth in Sambrook et al (see attached pages from the 1989 edition of Sambrook et al, including the last two pages which disclose the formula for calculating T_m) would certainly be known by, and readily available to, one of ordinary skill in the art. Further, Tomasetto, cited by the Examiner as teaching a protein identical to SEQ ID NO 1 minus the putative signal peptide, discloses the selective hybridization of an HSP probe to genomic DNA in a Southern blot (see Figure 7 of Tomasetto) under the conditions set forth in the protocol for Southern hybridization provided on page 413 of Tomasetto. Thus, in view of the teachings in the specification and the knowledge in the art, it is Applicants' position that one skilled in the art

1 The claim in the Guidelines reads as follows: "An isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 1, wherein said nucleic acid encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity."

would recognize that consistent with Example 9 of the Written Description Guidelines, the inventors in the present application were in possession of the claimed invention at the time of filing of the present application.

Accordingly, in view of the above arguments and the amendments to claim 27 presented herein, Applicants submit that the amended claims are in compliance with the requirements of 35 USC § 112, first paragraph and withdrawal of this rejection is therefore respectfully requested.

Rejections Under 35 U.S.C. 112, second paragraph

The Examiner rejected the claims as indefinite over the use of the term "high stringency conditions" and "homologue".

Applicants respectfully traverse these rejections.

With respect to "high stringency conditions", the Examiner asserted that since the specification fails to precisely define "stringent conditions", any degree of stringency is embraced by the claims.

In reply, Applicants note that the specification in disclosing the term "high stringency conditions" refers to Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY , 1989 (page 3, lines 5-11 of application), a well known handbook of molecular biology. As it is well settled that Applicants need not disclose what is well-known in the art [see, for example, In re Wands 8 USPQ2d 1400, 1402 (Fed. Cir. 1988) and the determination of hybridization and wash conditions based on the calculated T_m of the hybrid formed between a probe and its target are set forth in the pages of Sambrook et al attached in response to the section 112, first paragraph rejection (see, in particular, the last two pages of the attachment from Sambrook et al), Applicants submit that the phrase "high stringency conditions" would have a clear and definite meaning to one of skill in the art.

Turning to the term "homologue", the Examiner asserts that this term is indefinite

because one cannot "determine what additional or material functional limitations are placed upon a claim by the presence of this element." (page 7 of Office Action). In reply, Applicants submit that this rejection is rendered moot by the additional limitations placed on the term homologue in amended claim 27.

Thus, in view of the above amendments and remarks, withdrawal of the rejections under 35 U.S.C. 112, second paragraph is respectfully requested.

Rejections Under 35 U.S.C. 103(a)

A. The Examiner maintained the rejection of 27-36 claims as obvious over Onda in view of Tomasetto, Alberts, Hitzeman and Lodish and applied the rejection to added claim 39.

The Examiner, citing to Onda's expression in yeast of a polypeptide with high homology to PSP, suggests that one would have been motivated to choose yeast as the expression system for hSP and that the glycosylation of of hSP "would flow naturally from following the teachings of Onda regarding expression in yeast" (page 5 of Office Action).

Applicants respectfully traverse this rejection.

For the following reasons, Applicants submit that it would not have been obvious in view of the cited art to select yeast as the host cell for the recombinant expression of the hSP protein as recited in the pending claims.

First, at the time of filing of the present application, it was clearly understood by those skilled in the art that posttranslational processing of sugar moieties on proteins expressed in mammalian cells (ie the source of human spasmolytic polypeptide) differed from that in yeast and that expression of a mammalian protein in yeast (as opposed to mammalian cells) could result in changes to the glycosylation pattern of the polypeptide that could affect its proper folding, secretion and biological activity in an unpredictable manner[2]. For example, in a review article by Romanos et al on foreign gene expression in yeast [Yeast (1992) 8:423-488,

2 Of course, this is assuming arguendo that one skilled in the art would have even chosen expression in a host cell which allows post-translational modifications over the more cost effective bacterial expression since it was not known at the time of filing of the present application whether hSP was glycosylated.

copy attached], the authors, in discussing the secretion of foreign proteins from yeast and the modifications to glycosyl structures that take place in the Golgi, state:

"These modifications differ from those made by higher eucaryotic cells and, as a result, glycosylation is increasingly regarded as a major drawback to the secretion of therapeutic glycoproteins from yeast" (page 445, right-hand column, emphasis added).

The authors further state:

"Glycosylation is both organism and cell-type specific and therefore expression of a protein in a heterologous system will almost certainly result in a product with different modifications from the native material. This may affect the function or immunogenicity of the protein" (paragraph bridging pages 448-449).

The above statements are supported by the Hitzeman et al [Methods in Enzymology, 185: 421-440] article cited by the Examiner in which the authors state that "there is a gross difference between the sugar content of a heterologous protein produced in yeast and mammalian cells" (sentence bridging pages 436-437) and disclose that gp120 expressed in yeast is hyperglycosylated relative to mammalian gp120 and that the hyperglycosylated gp120 does not bind to the CD4 receptor (page 438). Thus, it is Applicants' position that one skilled in the art wishing to recombinantly express a mammalian protein such as hSP at the time of filing of the present application would be motivated by the teachings in the literature to select mammalian cells as the host rather than yeast[3].

Indeed, Applicants' position is fully supported by, and consistent with, the position taken by the Patent Office during the prosecution of priority application 08/491,796, where the Patent Office cited to the aforementioned Hitzeman et al article as teaching "the desirability of choosing a mammalian host over yeast for the purpose of providing glycosylation closest to that found in the natively expressed protein" (page 8 of January 13, 1997 Office Action).

Moreover, Applicants submit that in addition to the differences in glycosylation between mammalian cells and yeast, one skilled in the art at the time of filing of the present application would not have reasonably expected that a polypeptide

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3 Since the 60 amino acid pSP protein of Onda, in contrast to the 106 amino acid hSP protein, does not contain a putative N-linked glycosylation site, such considerations would not have been an issue for Onda.

the size of hSP could be expressed and secreted from yeast. For example, the aforementioned 1992 Romanos et al publication disclosed that the secretion of heterologous proteins from yeast had been most successful with peptides such as EGF (54 amino acids) and insulin (51 amino acids) (ie peptides comparable in size to the 60 amino acid pSP of Onda) but that "the secretion of large proteins has proven less predictable"[4] (first paragraph of page 446). Thus, it is Applicants' position that Onda's expression in yeast of the 60 amino acid pSP would not have led one to expect that the 106 amino acid hSP could also be expressed and secreted from yeast. Indeed, the attached 1986 publication by Thim et al [Proc. Natl. Acad. Sci. USA , 83:6766-6770, copy attached] discloses that while the 90 amino acid preproinsulin could not be successfully expressed and secreted from yeast, analogs of shorter length were readily expressed and secreted. By comparison, the 406 amino acid human Factor VII polypeptide was readily expressed and secreted from a mammalian cell line (baby hamster kidney cells) [Thim et al (1988) Biochemistry, 27:7785-7793, copy attached].

Thus, in view of the difficulties associated with secreting polypeptides the size of hSP from yeast and the art recognized desirability of choosing a mammalian host over yeast for the purpose of providing glycosylation closest to that found in a natively expressed mammalian protein, Applicants submit that it would not have been obvious to recombinantly express hSP in yeast.

B. The Examiner also maintained the rejection of claims 27 and 36 as being unpatentable over Onda in view of Tomasetto, Alberts, Hitzeman and Lodish as applied to claim 27 and further in view of Turco.

In particular, the Examiner alleged that it would have been obvious to make a glycosylated hSP as taught by Onda in view of Tomasetto, Alberts, Hitzeman and Lodish and to modify that teaching by making a pharmaceutical composition as taught by Turco with a reasonable expectation of success. With all due respect, Applicants disagree.

As discussed above, one of ordinary skill in the art would not have had a reasonable expectation that a protein of the size of hSP could be successfully expressed

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4 The 3 references cited by Romanos (references 186,284,388) as "notable successes" in the secretion of large proteins from yeast all involved the expression of non-mammalian proteins.

and secreted from yeast and further, would have been motivated to use mammalian cells rather than yeast to obtain glycosylation closest to that found in the natively expressed hSP. Accordingly, as the prior art taught away from using yeast as the host cell for the recombinant expression of hSP, one would not have been motivated to make a pharmaceutical composition as taught by Turco and withdrawal of this rejection is therefore respectfully requested.

Obviousness-Type Double Patenting Rejection

The Examiner rejected claims 27-33 and 36 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-8 and 10-13 of US Patent No. 5,783,416.

In reply, Applicants submit that they will submit an appropriate terminal disclaimer to obviate this rejection upon indication of allowable subject matter by the Examiner.

The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

Date: August 19, 2002

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"Marked-Up" Copy of Amendments to the Specification

Please replace the paragraph at page 13, line 25 to page 14, line 6 with the following paragraph:

--The 115 bp PCR fragment was digested with DdeI and then ligated to a 31 bp duplex formed from the oligonucleotides (GAGAAACCCTCCCCCTGCCAGTGCTCCAGGC) (SEQ ID NO:6) and (TCAGCCTGGAGCACTGGCAGGGGGAGGGTTTCTC) (SEQ ID NO:7). The ligation product was amplified by PCR using forward primer 3 (GCTGAGAGATTGGAGAAGAGAGAGAAACCCTCCCCCT) (SEQ ID NO:[7] 8) and reverse primer 2. The 3' part of primer 3 is identical to the N-terminal encoding part of the HSP gene and the 5' part of primer 3 is identical to the C-terminal encoding part of the hybrid leader gene (Fig. 3). In-frame fusion of the hybrid leader gene and the first trefoil domain from HSP was obtained by overlay extension PCR [31]. The product was digested with EcoRI and AvaII and isolated as a 360 bp DNA fragment.--

Please replace the paragraph at page 14, line 7 to page 14, line 25 with the following paragraph:

--The second trefoil domain of HSP was PCR-cloned from human genomic DNA as described for the first domain by replacing primers 1 and 2 with forward primer 4 (TGCGTCATGGAGGTCTC) (SEQ ID NO:[8] 9) and reverse primer 5 (AGCACCATGGCACTTCAAAG) (SEQ ID NO:[9] 10) (Fig. 3). Reverse primer 5 introduces a NcoI site as a silent mutation. The PCR product, a 115 bp fragment, was isolated and digested with DdeI and NcoI resulting in a 91 bp fragment. To this fragment were ligated two synthetic duplexes. The first, encoding the amino acid sequence between the two trefoil domains, consisted of the oligonucleotides (GTCCCCTGGTGTTTCCACCCCCTCCCAAAGCAAGAGTCGGATCAGTGCGTCATGGAGGTC) (SEQ ID NO:[10] 11) and (TGAGACCTCCATGACGCACTGATCCGACTCTTGCTTTGGGAGGGGGTGGAAACACCAGGG) (SEQ ID NO:[11] 12). The second, a 46 bp NcoI - XbaI fragment encoding the C-terminal part of HSP, consisted of the oligonucleotides (CATGGTGCTTCTTCCCGAACTCTGTGGAAGACTGCCATTACTAAGT) (SEQ ID NO:[12] 13) and (CTAGACTTAGTAATGGCAGTCTTCCACAGAGTTCGGGAAGAAGCAC) (SEQ ID NO:[13] 14). After AvaII digestion a 195 bp AvaII - XbaI fragment was isolated.--

"Marked-Up" Version Of Amendments To The Claims

27. (Twice Amended) An isolated human spasmolytic polypeptide having an amino acid sequence according to SEQ ID NO:1

Glu Lys Pro Ser Pro Cys Gln Cys Ser Arg Leu Ser Pro His Asn Arg Thr Asn Cys Gly Phe Pro Gly Ile Thr Ser Asp Gln Cys Phe Asp Asn Gly Cys Cys Phe Asp Ser Ser Val Thr Gly Val Pro Trp Cys Phe His Pro Leu Pro Lys Gln Glu Ser Asp Gln Cys Val Met Glu Val Ser Asp Arg Arg Asn Cys Gly Tyr Pro Gly Ile Ser Pro Glu Glu Cys Ala Ser Arg Lys Cys Cys Phe Ser Asn Phe Ile Phe Glu Val Pro Trp Cys Phe Phe Pro Asn Ser Val Glu Asp Cys His Tyr

or a homologue thereof [comprising] that

A) has [at least] six disulphide bonds that form two trefoil domains, where the 12 cysteines that form the six disulphide bonds are in the configuration 1-5, 2-4, 3-6, 7-11, 8-10 and 9-12, and

B) is encoded by a nucleic acid sequence that hybridizes under high stringency conditions to a nucleic acid sequence that encodes SEQ ID NO:1,

wherein said polypeptide is characterized by being in N-glycosylated form and having spasmolytic activity.